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## A PROCESS OF TEMPORARY CHAIN FORMATION BY FRONTONIA.<sup>1</sup>

WALDO SHUMWAY.

In May of this year, while studying the protozoan fauna of a small pond in the New York Botanical Gardens, the writer observed what appeared to be a chain of four holophrya-like ciliates. A careful search of the culture made during the following two weeks revealed about a dozen more such chains before the species disappeared. During this time the writer attempted to obtain a pure culture of these interesting forms, with an idea to studying the nuclear changes involved. Although these attempts were all, ultimately, unsuccessful, it has seemed best to put on record what few facts have been observed. The data here given has been obtained from the study of the living material and some few preparations both *in toto* and sectioned.

The following stages in the formation of these chains have been observed; single individuals, two-cell chains and four-cell chains. No chains of three or more than four cells have been observed among the large number (over one hundred and fifty) observed.

The solitary individual is an exceedingly large (circa 300 micra) ovoid holotrichous ciliate, densely pigmented and filled with large alveoli, of some alloplasmic substance which stains deeply with nuclear dyes. The color is dark brown to black with transmitted light and light brown to white with reflected light. The mouth is anterior and lateral with rows of cilia which simulate two undulating membranes. There is a large lateral contracting vacuole. The cortical layer contains trichocysts. The macronucleus is large and oval, the micronuclei have not been observed in my material. The form is an active swimmer in the surface film of a large culture, but when isolated in Syracuse watch glasses sinks to the bottom and encysts. Details of this process are given below.

In cases where the individual neither dies nor encysts after

<sup>1</sup> From the Zoölogical Laboratory, Columbia University.

isolation, it becomes transformed by clearing of the pigment, lengthening and flattening the general shape of the body until it resembles the well-known species *Frontonia leucas*. To this species, therefore, I have assigned my material, although I am not positive whether what I have observed is a new stage in the life-cycle of *Frontonia leucas* or a new species.

The process of chain formation is inaugurated by the formation of a large transparent cyst through the exudation of some gelatinous material in which the surrounding zoöglœa becomes entangled in large quantities. Within this cyst the individual slowly rotates, all the cilia beating and the contractile vacuole pulsating regularly. In one individual followed under the microscope a single transverse division occurred about forty-five minutes after the beginning of encystment, and half an hour from its completion. Nine hours after, the daughter cells were dead, still connected and inside the cyst wall.

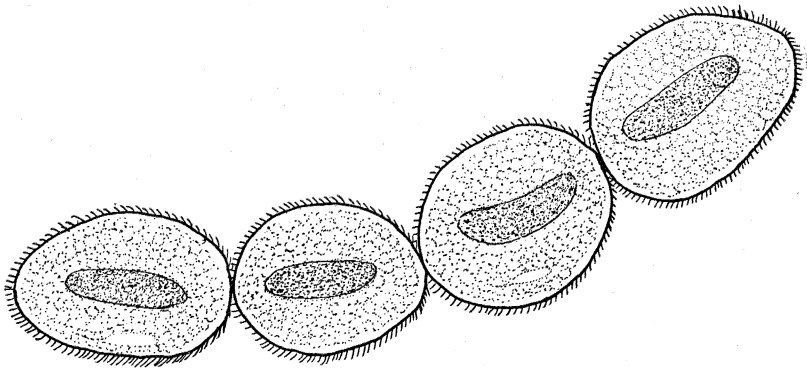


FIG. 1. Chain of four cells, *Frontonia leucas*. 200 X. Outline drawn with camera from preparation fixed with sublimate-acetic and stained with picrocarmine. Details restored from free-hand drawings of living material.

Another encysted individual which had been isolated at the same time but which unfortunately had not been followed under the microscope had formed a chain of four cells. The writer has at different times observed in these cysts, single, double, and quadruple forms, as well as cysts containing two or four separate cells. One chain of four individuals was removed from its cyst for observation: it swam about for a time resembling an animated chain of beads, but finally broke up into its four

constituent parts, which shortly assumed the typical *Frontonia leucas* appearance.

While the writer has not actually observed the process of transformation from the two-cell to the four-cell stage assumed above, he feels convinced from the facts cited as well as from sectioned material in which the macronuclei of the two anterior and the two posterior cells seem to have just undergone division, that these chains are formed by two successive transverse divisions without separation of the daughter cells and not by a single quadrupartite or by three successive terminal divisions as might be argued from *a priori* grounds.

Division preceded by encystment is not unknown among the free-swimming ciliates. In *Otostoma carteri* and *Amphileptus meleagris* according to Saville-Kent the divisions are sometimes multiple, but the daughter cells separate immediately after each division.

Chain formation too is not unknown among the ciliates, although heretofore it has been observed only among the astomatous forms parasitic in the digestive tracts of vertebrates. In these parasites, however, the chains are often of great length and are formed, so the weight of evidence appears, by a process of terminal budding. For an excellent discussion of these forms see Cépède (1910).

Jennings ('08) records a strain of *Paramæcia* appearing in his cultures which had apparently lost the power of separation after division. The general appearance and weak vitality of these forms, however point to the conclusion that Jennings was dealing with a weakened pathological race and not a genetic mutation.

The frequency with which these chains appeared, nearly ten per cent. of all the *Frontonia* observed, leads the writer to conclude that the phenomena described in this paper form part of a normal method of reproduction.

I have made attempts to raise these forms on hay infusion, rotten lettuce infusion (recommended by Popoff, '08), thyroid extract and the filtered water of the medium in which they were discovered. All were unsuccessful. I have been unable to maintain isolated individuals in Syracuse glasses of their

normal medium longer than two days. For this reason I am unable to give a fuller account of the process under discussion nor carry out the experimental work suggested by it. The case however appears to be a unique instance of normal temporary chain formation among the free-living ciliates.

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